SYNTHESIS OF SUGAR-AMINO ACID MODEL COMPOUNDS

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In the course of the synthesis of 6-amino-6-deoxy-1,2-3,4-di-O-isopropylidene-D-galactose, tri-(6-deoxy-1,2-3,4-di-O-isopropylidene-D-galactose) hydrazine was also obtained.

Reactions of 6-amino-6-deoxy-1,2-3,4-di-O-isopropylidene-D-galactose with O-tetrahydropyran-2-yl-N-benzyl sulphonyl-DL-serine and N-benzyl sulphonyl glycine, in presence of dicyclo-hexyl carbodi/imide have been described.

Introduction

In order to gain some insight into the nature of various possible linkages, which can theoretically occur between a carbohydrate and a protein in glycoproteins and mucopolysaccharides, syntheses and studies of a large number of model compounds have recently been undertaken. In many such cases reported in the literature, 2-amino-2-deoxy-D-glucose has been condensed with *N*-acetylated amino acids or peptides with the formation of an amide bond. [1–4] A similar reaction involving the amino groups of amino sugars other than 2-amino-2-deoxy-D-glucose has hitherto been rarely investigated. We have therefore synthesized 6-amino-6-deoxy-1,2-3,4-di-*O*-isopropylidene-D-galactose and condensed it with *O*-tetrahydropyran-2-yl-*N*-benzyl sulphonyl-DL-serine.[5] A similar reaction using *N*-benzyl sulphonyl glycine[6] was also investigated.

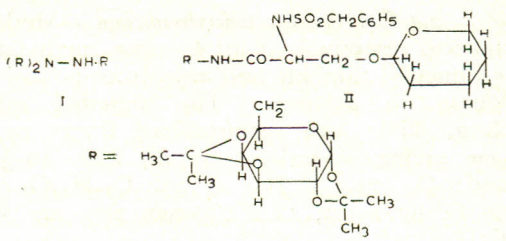
Synthesis of 6-amino-6-deoxy-di-*O*-isopropylidene-D-galactose was reported -1,2-3,4 by Freudenberg and Doser,[7] who treated 1,2-3,4-di-*O*-isopropylidene-6-*O*-tosyl-D-galactose with ammonia to obtain the amino sugar. Thereafter, the isopropylidene groups were removed but the resulting product could not be crystallised. More recently an improved method of synthesis has been described by Veksler.[8] In the present investigation, however, the general method of Wolfrom and Shafizadeh[9] has been followed.

Experimental

Synthesis of 6-amino-6-deoxy-1,2-3,4-di-O-isopropylidene-D-galactose.—A solution of 1,2-3,4-di-*O*-isopropylidene-6-*O*-tosyl-D-galactose[10] (18.6 g) in anhydrous hydrazine (220 ml) was refluxed at 145° for 24 hr with the exclusion of atmospheric moisture. The syrupy product obtained by removal of excess of hydrazine, was taken in ethanol and stirred with Raney nickel to remove traces of hydrazine. The hydrazino derivative obtained after filtration of Raney nickel, was reduced in a Parr apparatus at 3 atmospheric pressures of hydrogen for 24 hr using Raney nickel as catalyst. The reaction mixture was filtered and the filtrate on concentration yielded (4.1 g) 6-amino-6-deoxy-1,2-3,4-di-*O*-isopropylidene-D-galactopyranose toluene-*p*-sulphonate, m.p. 190-192° $[\alpha]_D^{21}$ −36° (*c* 1.04 in chloroform). (Found: C,52.5; H, 6.6; N, 3.4. $C_{19}H_{29}NO_8S$ requires: C, 52.8; H, 6.75; N, 3.2%.) Veksler[8] reports m.p. 182°, $[\alpha]_D^{25}$ −31° for this compound.

The free amino sugar was obtained by shaking 6-amino-6-deoxy 1,2-8,4-di-*O*-isopropylidene-D-galactopyranose toluene-*p*-sulphonate in chloroform with 50% potassium hydroxide solution. The organic phase was separated, dried and concentrated to yield a syrup.

In a repeat synthesis as above starting with 8.2 g of 1,2-3,4-di-*O*-isopropylidene-6-*O*-tosyl-D-galactose, after the isolation of 6-amino-6-deoxy-1,2-3,4-di-*O*-isopropylidene-D-galactopyranose toluene-*p*-sulphonate, a second crop on recrystallisation had a melting point 190–191° $[\alpha]_D^{29}$ −100° (*c*, 0.72 in methanol). This product did not show a positive ninhydrin reaction. Elemental analysis and molecular weight determination



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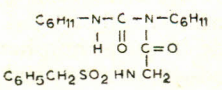

III

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(modified Barger's method)[11] showed it to be tri (6-deoxy-1, 2-3,4-di-*O*-isopropylidene-D-galactose) hydrazine (compound I) (Found: C, 56.9; H, 7.45; N, 3.7; mol. wt. 788. $C_{36}H_{58}N_2O_{15}$ requires: C, 57.0; H, 7.7; N, 3.7%; mol. wt. 759.) Corbett and Winters[12] report m.p. 196.5–198.5° for this compound. The IR spectrum of this material kindly provided by Dr. Corbett, was indistinguishable from the product isolated as above.

Preparation of 6-(*O-tetrahydropyran-2-yl-N-benzyl sulphonyl-*DL*-serinyl*) *amino*-6-*deoxy*-1,2-3,4-*di*-*O Isopropylidene*-D-*galactopyranose*.—The condensation of 6-amino-6-deoxy-di-*O*-isopropylidene-D-galactose with *O*-tetrahydropyran-2-yl-*N*-benzyl sulphonyl-DL-serine was effected by the use of dicyclohexyl carbodiimide. To a stirred solution of 6-amino-6-deoxy-di-*O*-isopropylidene-D-galactose (0.76 g) in dry ether (50 ml) were added dicyclohexyl carbodiimide (0.69 g) and *O*-tetrahydropyran-2-yl-*N*-benzyl sulphonyl-DL-serine (1.28 g) in ethyl acetate (30 ml). After stirring at room temperature for 10 hr glacial acetic acid (3 drops) were added, *N*,*N*′-dicyclohexylurea was removed by filtration and the filtrate concentrated to a syrup (1.5 g). The syrup was taken in ether and washed successively with 0.2N hydrochloric acid (3 ml), water, 0.2N sodium bicarbonate (3 ml), water and dried. The optically inactive product crystallised out from ether by slow evaporation, m.p. 88–91° (compound II). (Found: C, 55.3; H, 7.2; N, 4.9; $C_{27}H_{40}N_2O_{10}S$ requires: C, 55.5; H, 6.9; N, 4.8%).

An attempt to remove the *N*-benzylsulphonyl group from the above compound by reduction with sodium in liquid ammonia,[6] yielded a product which showed a single spot on paper chromatography in ethanol–butanol–water (1:4:5 v/v) with ninhydrin. However, it failed to crystallise.

Attempted condensation of 6-*amino*-6-*deoxy*-1,2-3,4-*di*-*O*-*isopropylidene*-D-*galactose with N-benzyl sulphonyl glycine*.—To a solution of 6-amino-6-deoxy-1,2-3,4-di-*O*-isopropylidene-D-galactose (0.45 g) in a mixture of dry ether (25 ml) and dry ethanol (25 ml) were added *N*-benzylsulphonylglycine (0.09 g) and dicyclohexylcarbodiimide (1.3 g), and the mixture stirred at room temperature for 12 hr. Unreacted *N*-benzylsulphonylglycine and the precipitated *N*,*N*′-dicyclohexylurea were filtered off and the filtrate concentrated to yield a syrup (2.13 g). The product which crystallised from the syrup in ethanol after a week had m.p. 158–159°. Glycine could not be detected on paper chromatograms (ethanol–butanol–water 1:4:5 v/v), after acidic hydrolysis (2N sulphuric acid for 25 hr at 100°) of this product. However, two spots indistinguishable in mobility from those of *N*-benzylsulphonylglycine and *N*,*N*-dicyclohexylurea were revealed. (Found: C, 60.5; H, 7.3; N, 9.8. $C_{22}H_{33}N_3O_4S$ requires: C, 60.7; H, 7.3; N, 9.8%) compound III.

Discussion

Since the introduction of dicyclohexyl-carbodimiide as a condensing agent by Sheehan and Hess,[13] and Khorana[14] in 1955, it has found many useful applications in peptide synthesis. It enabled the reaction of an *N*-acylated amino acid to be carried out directly at room temperature with an amino acid ester, with the formation of a protected dipeptide. Following the cleavage of the blocking groups, the pure di-or tripeptides are easily obtained. The bi-product of the reaction *N*,*N*′-dicyclohexyl urea is insoluble in most common organic solvents and hence can be readily removed. The foregoing procedure is equally applicable to the formation of sugar-amino acid compounds, when both these components have suitably protected functional groups. Such reactions are best carried out in inert solvents like methylene chloride, although aqueous pyridine has also been employed for this purpose. The use of an aqueous solvent, however, is known to effect the course of the reaction.[1]

In the present work the easy solubility of *O*-tetrahydropyran-2-yl-*N*-benzylsulphonyl-DL-serine in ether made it specially suitable for reaction with 6-amino-6-deoxy-1,2-3,4-di-*O*-isopropylidene-D-galactose, using dicyclohexylcarbodiimide as the coupling agent. Subsequent attempts, following the isolation of the pure product, to remove the amino-protecting benzylsulphonyl group by the action of sodium in liquid ammonia, yielded a syrupy mixture which did not crystallise. The oxalate of the expected 6-(*O*-tetrahydropyran-2yl-DL-serinyl) amino-6-deoxy-1,2-3,4-di-*O*-isopropylidene-D-galactose also could not be fractionally crystallised from this mixture.

Because of the very poor solubility of *N*-benzylsulphonylglycine in ether or ethyl acetate, its reaction with 6-amino-6-deoxy-1,2-3,4-di-*O*-isopropylidene-D-galactose could only be investigated in a mixture of ether and dry ethanol. An optically inactive product thus obtained crystallised out of the reaction mixture after a week. It was hydrolysed and the hydrolysate neutralised and applied on a paper chromatogram. Two spots corresponding in mobility to *N*-benzylsulphonylglycine and *N*,*N*′-dicyclohexylurea could be detected by potassium iodide–starch spray.[15] This evidence together with elemental analyses showed that the

substance so obtained was a reaction product of *N*-benzylsulphonyl-glycine and *N*,*N*′-dicyclohexylurea. It would be noteworthy that Ichiro Muramatsu[16] found that in the preparation of bis(*N*-phthaloyl-β-alanine) anhydride, *N*-(*N*″-phthaloyl-β-alanyl)-*N*,*N*′-dicyclohexylurea was also formed. He presented evidence to show that the formation of ureides must proceed through the initial addition of —COOH group to *N*,*N*′-dicyclohexylcarbodiimide followed by rearrangement. Similarly Rydon[17] has also mentioned the possible formation of *N*-acylurea derivatives from the carboxyl components, while using dicyclohexylcarbodiimide as a coupling reagent.

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References

1. N.K. Kochetkov, V.A. Dervitskaya, L.H. Likhosherstov, N.V. Molodtsov and S.G. Kara Murza, Tetrahedron, **18,** 273 (1962).
2. J.K.N. Jones, M.B. Perry, B. Shelton and D.J. Walton, Canad. J. Chem., **39,** 1005 (1961).
3. J.K.N. Jones, J.P. Millington and M.B. Perry, Canad. J. Chem., **40,** 2229 (1962).
4. S.M. Amir, J.S. Brimacombe and M. Stacey, Nature, **203,** 401 (1964).
5. S.M. Amir, M.Z. Atassi, S.A. Barker, J.S. Brimacombe and M.C. Keith, Nature, **192,** 1069 (1961).
6. H.B. Milne and C.H. Peng, J. Am. Chem. Soc., **79,** 639, (1957).
7. K. Freudenberg and A. Doser, Ber., **58,** 294 (1925).
8. V.I. Veksler, Zhur. Obshchei Khim., **31,** 989, (1961).
9. M.L. Wolfrom, F. Shafizadeh, R.K. Armstrong and M.T. Shen Han, J. Am. Chem. Soc., **81,** 3716 (1959).
10. A.L. Raymond and E.F. Schroeder, J. Am. Chem. Soc., **70,** 2785, (1948).
11. J.B. Niederl, D.R. Kasanof, G.K. Kisch and D.S. Rao, Mikrochemie, 132 (1949).
12. W.M. Corbett and D. Winters, J. Chem. Soc., 4823 (1961).
13. J.C. Sheehan and G.P. Hess, J. Am. Chem. Soc., **77,** 1067 (1955).
14. H.G. Khorana, Chem. Ind., 1087 (1955).
15. H.N. Rydon and P.W.G. Smith, Nature., **169,** 922 (1952).
16. I. Muramatsu, Nippon Kagaku Zasshi, **82,** 83 (1961).
17. Raydon, Peptide Synthesis, Lecture Series **5,** 18 (1962).